

# Atherosclerosis and Cardiovascular Diseases in Progeroid Syndromes

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Hutchinson–Gilford progeria syndrome (HGPS) and Werner syndrome (WS) are two of the representative genetic progeroid syndromes and have been widely studied in the field of aging research. HGPS is a pediatric disease in which premature aging symptoms appear in early childhood, and death occurs at an average age of 14.5 years, mainly due to cardiovascular disease (CVD). Conversely, WS patients exhibit accelerated aging phenotypes after puberty and die in their 50s due to CVD and malignant tumors. Both diseases are models of human aging, leading to a better understanding of the aging-associated development of CVD. In this review, we discuss the pathogenesis and treatment of atherosclerotic diseases presented by both progeroid syndromes with the latest findings.

**Key words:** Hutchinson–Gilford progeria syndrome, Werner syndrome, Atherosclerosis, Cardiovascular disease, Aging

## Introduction

In 2020, the world's population aged 65 or older was 727 million. Over the next 30 years, the number of older people worldwide will be more than double and is projected to exceed 1.5 billion by 2050<sup>1)</sup>. Aging is an independent risk factor for the development of cardiovascular diseases (CVDs) and is considered to be the greatest risk<sup>2,3)</sup>. Therefore, the research focused on the mechanism underlying aging-associated CVD development is essential.

Progeroid syndrome represented by Hutchinson–Gilford progeria syndrome (HGPS) and Werner syndrome (WS) has been studied as a model disease of human aging because aging-like symptoms appear from a young age and their pathological condition mimics general aging. These premature aging syndromes also display the early onset of CVD. This paper outlines the clinical features and molecular mechanisms of the two aforementioned syndromes, focusing on atherosclerotic diseases, with the latest findings.

## Hutchinson–Gilford Progeria Syndrome

HGPS is an ultra-rare autosomal dominant genetic premature aging syndrome that occurs in one in four to eight million births and causes death at an average age of 14.5 years due to myocardial infarction or stroke<sup>4,5)</sup> (**Table 1**). Because of the abnormal splicing of the *LMNA* gene on chromosome 1, the lamin A protein encoded by this gene cannot be normally produced, and an abnormal protein called progerin accumulates in the nucleus. The main symptoms are scleroderma-like skin, joint contractures, bone abnormalities, hair loss, growth retardation, and atherosclerotic diseases, such as myocardial infarction and stroke.

## Clinical Characteristics of HGPS

Infants with HGPS are normal at birth, but the mean weight is slightly small for gestational age<sup>4)</sup>. The first pathognomonic signs are prominent veins on the nose bridge, followed by growth retardation, hair loss, and reduced subcutaneous fat around 6 to 12 months, and the diagnosis is often made between 2 and 3 years

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Received: July 15, 2021 Accepted for publication: August 16, 2021

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**Advance Publication Journal of Atherosclerosis and Thrombosis**

**Accepted for publication: August 16, 2021 Published online: September 11, 2021**

**Table 1.** Summary of the characteristics of HGPS and WS

	HGPS	WS
Disease prevalence	1 in 20 million <sup>5)</sup>	9.0 in 1 million in Japan <sup>87)</sup> , 1.0 to 2.7 in 1 million globally <sup>47)</sup>
Ethnicity	Ubiquitous <sup>7)</sup>	Relatively prevalent in Japanese and Sardinian <sup>47)</sup>
Sex	Equally affected <sup>7)</sup>	Equally affected <sup>48)</sup>
Lifespan	Average 14.5 years <sup>5)</sup>	Average 55.0 years <sup>65)</sup> , median 54.3 years <sup>88)</sup>
Responsible gene and frequent mutation	<i>LMNA</i> : c.1824C>T (90% of patients) <sup>5)</sup>	<i>WRN</i> : c.3139-1G>C (70.7% of allele in Japanese patients), <i>WRN</i> : c.1105C>T (18.6% of allele in non-Japanese patients) <sup>47)</sup>
Diabetes or IGT	15.4% of patients <sup>6)</sup>	67.5% of patients <sup>67)</sup>
Dyslipidemia	71.4% of patients <sup>6)</sup>	65.0 to 85.0% of patients <sup>52, 67)</sup>
Hypertension	46.7% of patients <sup>6)</sup>	42.5% of patients <sup>67)</sup>
CVD	100% had adventitial thickening in the carotid artery, 18.2% had the low ankle-brachial index <sup>6)</sup>	15% had ASO, 2.5% had AP or MI, none had cerebral artery disease <sup>67)</sup>
Causes of death (percentage of total)	Heart failure (80%), head injury (9%), complications of surgery (4%), stroke (3%) <sup>7)</sup>	Malignancy (56%), AMI (28%), infection (14%), cerebral bleeding (2%) <sup>65)</sup>

Abbreviations: IGT, impaired glucose tolerance; ASO, atherosclerosis obliterans; AP, angina pectoris; MI, myocardial infarction; AMI, acute myocardial infarction.

of age. Symptoms in the craniofacial region gradually appear, including alopecia, with only a few hairs, abnormally prominent scalp veins, larger skull than the facial bone, pseudo-protrusion of the eyes due to a decrease in surrounding adipose tissue, loss of subcutaneous fat and muscles in the face, a narrowing of the nose bridge and a hooked nose, wrinkles around the lips due to thinning of the skin, abnormal dentition, and caries, a small jaw, large ears lacking earlobes, and a high-pitched voice. Symptoms in the trunk and extremities include loss of subcutaneous fat and muscle mass, protruding joints and decreased range of motion, and hypertrophy of the tips of the fingers. On the other hand, there is no intellectual impairment or psychiatric symptoms, and most of the affected children are charming and active. The growth disturbance is remarkable, especially in weight, with most children over 12 years old weighing around 15 kg (same as 3- to 4-year-old healthy children). As a result, they gradually develop the appearance resembling older people.

In a report of 15 white patients (median age, 6 years and 11 months), the average body fat percentage was as low as 16% (below -1 SD) and tended to decrease with age<sup>6)</sup>. Seven had higher blood pressure than healthy children of the same age and height, five had abnormal electrocardiograms, and three had echocardiographic abnormalities at rest. Carotid artery ultrasonography revealed adventitial thickening in all

patients and stenosis and occlusion in three. In addition, the ankle-brachial index decreased in three patients. Although muscle strength was preserved, 11 patients had osteoporosis, and all 15 patients had osteolysis of the distal phalanges and clavicles, as seen on X-ray. Blood tests revealed a decreased level of high-density lipoprotein cholesterol in 10 patients. Glycated hemoglobin was in the normal range, but five had elevated fasting insulin levels, and one was diagnosed with diabetes *via* oral glucose tolerance test. The creatinine and urea nitrogen levels were in the normal range.

The cause of death is predominantly CVD<sup>7)</sup>. CVD events do not occur until around the age of 5 years, but the children gradually suffer from dyspnea due to heart failure or ischemic heart disease and paralysis caused by stroke<sup>4, 8)</sup>. Myocardial infarction and heart failure are frequent and account for 80% of deaths<sup>4, 7)</sup>. Other reported causes of death include intracranial hemorrhage, seizures, infections, and complications from cardiovascular surgery<sup>4, 7)</sup>.

In autopsy, the loss of vascular smooth muscle in the media is prominent<sup>4, 9)</sup>. Calcification and plaque formation in the coronary arteries vary from patient to patient<sup>4, 10)</sup>.

A study that assessed 27 HGPS patients reported that diastolic dysfunction was the most prevalent cardiac abnormality<sup>11)</sup>. Other abnormalities, such as left ventricular (LV) hypertrophy, LV systolic

dysfunction, and valve disease, were less common in those below 10 years old compared with teenagers<sup>11</sup>. These findings are consistent with general aging-associated heart failure, which is attributed to an impairment of the diastolic filling of the left ventricle, rather than a dysfunction of systolic function<sup>12, 13</sup>. A case report of a 14-year-old girl with HGPS described dilated cardiomyopathy with congestive heart failure<sup>14</sup>. Previous reports revealed that progerin is upregulated in human hearts with dilated cardiomyopathy<sup>15, 16</sup>, implying causal relationships between progerin expression and pathogenesis of cardiomyopathy.

Although it is believed that there is no difference in the disease incidence rate and phenotypes by race<sup>7, 17</sup>, there are relatively few reports from Asia and Africa. Four Japanese and nine Chinese cases were well described by Sato-Kawano *et al.* and Wang *et al.*, respectively<sup>18, 19</sup>. Also, patients in Africa were reported by two case studies<sup>20, 21</sup>.

### Molecular Mechanism of Progerin Expression

Lamin is a constituent of the lamina, an intermediate filament responsible for the lining structure of the nuclear envelope. There are three types of lamin, namely, lamin A, lamin B, and lamin C. They are involved in the regulation of DNA transcription, replication, repair, and signal transduction from the cytoskeleton to the nucleus by stabilizing the structure of the nuclear envelope and anchoring chromosomes and transcription factors to the nuclear envelope<sup>22</sup>. Diseases caused by lamin abnormalities include HGPS, muscular dystrophy, neuropathy, and atypical WS<sup>23</sup>, which are collectively called laminopathies.

Lamin A/C is translated *via* alternative splicing from *LMNA*, which is located on the long arm of chromosome 1 and consists of 12 exons<sup>4</sup>. First, prelamin A is translated from *LMNA*. Prelamin A has a CAAX motif (C, cysteine; A, aliphatic amino acid; X, any amino acid) at the C-terminal, which works as an indicator for farnesyltransferase to farnesylate the cysteine residue. Next, Zmpste24, a metalloprotease, cleaves AAX. Then, the remaining cysteine undergoes methylation by isoprenylcysteine carboxyl methyltransferase, and the C-terminal 15 amino acids are removed by Zmpste24 and other endoproteases to form mature lamin A.

A point mutation (c.1824C>T, G608G, a silent mutation) has been found at codon 608 in the 11th exon of *LMNA* in almost all HGPS patients with classical symptoms. This results in an unusual splicing site and a loss of 50 amino acids, including the

Zmpste24 recognition site, which leads to the production of progerin, an aberrant protein that remains farnesylated.

Progerin acts in a dominant-negative manner<sup>24</sup>. Due to the affinity of the farnesylated portion to the membrane, it remains bound to the nuclear membrane even during the M phase of the cell cycle, thus inhibiting normal mitosis<sup>25</sup>. Nuclear accumulation of progerin causes abnormal nuclear morphology, dysregulation of other gene expressions, disruption of DNA repair mechanisms, shortening of telomeres, genomic instability, and abnormal mitochondrial function, leading to premature cellular senescence<sup>22</sup>. It has also been reported that H3K9 methylation is reduced in HGPS cells as in normal aging<sup>26</sup>. Interestingly, it is also known that progerin accumulates in cells of healthy older people<sup>27</sup>, and this discovery has brought further attention to HGPS as a model disease of aging.

### Cardiovascular Phenotypes in HGPS Mouse Models

HGPS patients develop severe vascular changes, primarily vascular smooth muscle cell depletion, calcification, and fibrosis, in addition to electrical and functional abnormalities in the heart. Several HGPS animal models have been created to recapitulate and analyze these phenotypes<sup>28</sup>.

The most widely used animal model is the *Lmna*<sup>G609G</sup> mouse<sup>29</sup>. Similar to HGPS patients, this mouse harbors a silent mutation resulting in abnormal splicing and production of progerin; moreover, it has shortened life span and bone abnormalities. Villa-Belosta *et al.* found that the aorta of *Lmna*<sup>G609G/+</sup> mice had excessive calcification and that there was insufficient production and extracellular accumulation of pyrophosphate, a major inhibitor of vascular calcification, in primary vascular smooth muscle cells of the aorta, leading to vascular calcification<sup>30</sup>.

On the other hand, *Lmna*<sup>G609G/G609G</sup> mice fed a high-fat diet do not develop atherosclerosis as observed in HGPS patients<sup>31</sup>. To address this issue, Hamczyk *et al.* generated *Apoe*<sup>-/-</sup>*Lmna*<sup>G609G/G609G</sup> mice and fed them with a high-fat diet<sup>32</sup>. They were able to develop atherosclerosis and recapitulate most of the cardiovascular phenotypes observed in HGPS patients.

The *Lmna*<sup>G609G</sup> mouse also reproduces the electrical phenotype in the heart. Macias *et al.* reported that cardiomyocytes from *Lmna*<sup>G609G</sup> mice show prolonged action potential duration and refractoriness after repolarization<sup>33</sup>, which may be related to the QT prolongation reported in some HGPS patients<sup>6</sup>.

## Treatment of HGPS

Currently, there is no fundamental treatment for HGPS. However, the most encouraging recent topic is the FDA's approval of lonafarnib, a drug that inhibits the farnesylation of lamin A<sup>34, 35</sup>. In an observational study with a median treatment duration of 2.2 years, 17 of 63 deaths occurred in the untreated group, compared with 4 of 63 deaths in the lonafarnib-treated group, with a hazard ratio of 0.23<sup>7</sup>. While lonafarnib improves cardiovascular phenotypes<sup>36</sup>, the addition of pravastatin and zoledronic acid has been found to be effective in treating musculoskeletal phenotypes<sup>37, 38</sup>.

CRISPR-based therapies are also in the limelight. Two reports of *Lmna*-targeted knockouts in *Lmna*<sup>G609G</sup> mice have observed prolonged lifespan, suppression of cardiac fibrosis, and improvement of vascular smooth muscle in the tunica media of the aortic arch<sup>39, 40</sup>. Interestingly, in both cases, the most efficiently genetically modified tissue was the liver, suggesting an association between hepatic dysfunction and the HGPS arteriosclerosis phenotype. In another report, mice carrying a mutation of a human HGPS patient (*LMNA*<sup>G608G</sup>) were repaired with a base editor, which substitutes A-T to G-C at c.1824 in *LMNA*<sup>41, 42</sup>. Although the most genetically repaired tissue was the liver like the above two reports, vascular smooth muscle restoration and adventitial fibrosis suppression were observed. The base editor has been shown to restore angiogenic potential by reducing progerin expression and increasing intracellular nitric oxide levels in HGPS-induced pluripotent stem (iPS) cell-derived endothelial cells *in vitro*<sup>43</sup>.

Osorio *et al.* has reported that treatment of *Lmna*<sup>G609G</sup> mice with morpholino oligos to suppress aberrant splicing leads to an extended lifespan<sup>29</sup>. Recently, it was reported that suppression of abnormal splicing in transgenic mice carrying the human *LMNA*<sup>G608G</sup> mutation could also result in up to 61.6% lifespan extension and inhibition of vascular smooth muscle loss in large vessels<sup>44, 45</sup>. It is epoch-making in that gene therapy can be performed without editing patients' DNA, and future clinical application is expected.

## Werner Syndrome

WS is an autosomal recessive progeroid syndrome, also known as adult progeria, due to the appearance of various premature aging symptoms in adulthood<sup>46</sup> (Table 1). The causative gene is WRN on the short arm of chromosome 8. About 70% of the cases reported to date are Japanese, and 1 in 150

normal Japanese people has a heterozygous mutation<sup>47</sup>. Patients with WS are generally short in stature but normally develop until puberty, and from around their 20s, they exhibit gray hair, hair loss, bird-like face, hoarseness, and scleroderma-like symptoms in the limbs<sup>48</sup>. Later, they develop cataracts, diabetes, osteoporosis, intractable skin ulcers, arteriosclerotic diseases, and malignant tumors (especially non-epithelial malignancy), and most of them die in their 50s.

## Clinical Characteristics of WS

One of the most important clinical features of the disease is intractable skin ulcers that occur mainly on the lower limbs, including the toes, heels, and Achilles tendons. In some cases, these ulcers lead to gangrene or osteomyelitis, leading to amputation of the lower extremities. Unlike foot ulcers of diabetes, WS ulcers are characterized by severe pain and subcutaneous calcification<sup>49</sup>, which significantly reduces the patient's quality of life.

In addition, WS patients often accumulate visceral fat, resulting in diabetes and dyslipidemia based on insulin resistance<sup>50</sup>. On the other hand, because the limbs wither and atrophy like branches, patients' body shapes are sometimes called Cushing-like appearance. The symptom of loss of muscle mass is called sarcopenia and has become a major topic in geriatrics. In a study of nine patients with WS, all of them met the diagnostic criteria for sarcopenia<sup>51</sup>.

The key signs to clinically suspect WS are the appearance of gray hair and hair loss (attention must be paid to hair dye and wigs) as well as bilateral cataracts under the age of 30. If multiple corn/callus and calcification of the Achilles tendon are observed, genetic testing should be conducted to confirm the diagnosis. Please refer to the "Management guideline for Werner syndrome 2020" series for the main strategy to treat each specific symptom<sup>52-59</sup>.

## Molecular Mechanism of WS

The WRN protein encoded by the *WRN* gene is a member of the RecQ helicase family together with RecQ1, BLM, RecQ4, and RecQ5 and has DNA helicase activity (the ability to unwind a DNA duplex into a single strand) as well as DNA exonuclease activity<sup>60</sup>. So far, at least 83 mutations have been reported<sup>47</sup>, and several new mutations have been identified in our laboratory<sup>61</sup>. On the other hand, there is no clear correlation between mutation sites and clinical signs.

WRN has a variety of functions, including DNA

replication and repair, telomere maintenance, and epigenetic regulation, such as heterochromatinization. Among them, many reports have confirmed the involvement of WRN in DNA double-strand break (DSB) repair. There are two main pathways in DSB repair, namely, non-homologous end joining (NHEJ) and homologous recombination, and the WRN protein is involved in both<sup>62</sup>. A recent report demonstrated that WRN proteins promote classical-NHEJ and inhibit alternative-NHEJ, leading to repair error suppression<sup>63</sup>.

In another study, Zhang *et al.* reported that the WRN protein is involved in the methylation of H3K9, a marker of heterochromatin<sup>64</sup>. This loss of methylation is also observed in a general aging process, suggesting that WS mimics the general aging from an epigenetic point of view.

In WS, accumulation of DNA mutations, shortening of telomeres, and abnormalities in histone methylation are assumed to be responsible for various pathological conditions. However, the causal relationship between these abnormalities at the molecular level and the pathological conditions remains to be elucidated, and further research is needed.

### Atherosclerotic Diseases in WS

Atherosclerotic disease is the second leading cause of death in WS<sup>65</sup>. According to a 2012 report of Japanese patients, the prevalence of vascular disease in WS was 1.1% for cerebral hemorrhage, 2.7% for cerebral infarction, 10.3% for angina and myocardial infarction, and 17.3% for arteriosclerosis obliterans<sup>66</sup>. The latest report from the Japanese WS Registry revealed a decrease in the prevalence of 0%, 0%, 2.5%, and 15%, respectively<sup>67</sup>. In addition to the use of statins<sup>68</sup>, this reduction may be due to the remarkable development of antidiabetic drugs in recent years<sup>67</sup>. In fact, in some of the case reports of WS in recent years, no obvious atherosclerotic disease was found<sup>69-72</sup> or was only slightly present<sup>61, 73, 74</sup>. In the case of a patient who died at 76 years of age, there were almost no findings of atherosclerosis in the cerebral arteries, and only calcification without stenosis was observed in the aorta and coronary arteries<sup>75, 76</sup>. In eight genetically diagnosed Chinese WS patients, the atherosclerotic disease was evident in only one patient<sup>77</sup>.

Dyslipidemia affects 65% to 85% of WS patients<sup>52, 67</sup>. Of those, the frequency of hypertriglyceridemia is somewhat higher. In fact, patients with hypertriglyceridemia accompanied by 3900 mg/dl of triglycerides had advanced three-vessel

disease requiring coronary artery bypass surgery<sup>78</sup>. In addition to statins to hypercholesterolemia, adequate management of hypertriglyceridemia may also be required.

On the other hand, in some cases, severe aortic stenosis, without the requirement of coronary intervention, has become a problem<sup>79, 80</sup>. In addition, cases of heart failure due to impaired coronary microcirculation with no coronary artery stenosis have been reported<sup>81</sup>. These suggest that arteriosclerosis in WS needs to be comprehensively evaluated.

A mouse model of WS does not develop arteriosclerosis<sup>82</sup>. A report of knockdown of the WRN gene using human endothelial cells *in vitro* demonstrated increased expression of inflammation and adhesion molecules<sup>83</sup>. On the other hand, a report of differentiation of WRN knockout embryonic stem cells into endothelial cells described no phenotypic changes<sup>84</sup>. Understanding the molecular mechanisms of atherosclerosis in WS requires further investigation.

### Conclusion

As aforementioned, HGPS and WS are representative diseases of premature aging, and patients suffer from shortened life expectancy and reduced quality of life. One reason for that is because the full picture of their underlying mechanisms is still unclear. Furthermore, we are globally encountering a super-aging society. The study of progeria is extremely significant as it will contribute to the elucidation of the mechanisms of a general aging. The registration of patients into the HGPS and WS registries both in Japan and overseas is currently ongoing, and we expect that the understanding of the natural history of the disease will elucidate the clinical problems that need to be addressed. In addition, analyses using primary patient samples and patient-derived iPSCs are underway, including in our laboratory, using state-of-the-art technologies, such as the genome, transcriptome, and epigenome analyses<sup>85, 86</sup>. These studies are expected to lead to breakthroughs in the treatment of progeria and the study of general aging.

### Funding

This work was supported by the Japan Society for the Promotion of Science (JSPS) KAKENHI under Grant Numbers JP20K16542 (H.K.) and JP20H03572 (Y.M.).

## Conflicts of Interest

The authors declare there are no conflicts of interest related to this work.

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